Claims

1. A culture method for producing a defined enzyme mixture and/or metabolite mixture optimized for the fermentation of one or more target substrates by contacting an inoculating mixed culture of microorganisms in a solid-phase bioreactor with one or more target substrates, one or more inducer substrates or any combination of target and inducer substrates, and by keeping the mixed culture under an appropriate selection pressure by a suitable selection of the culturing parameters and by a specific induction by the target and/or inducer substrate and/or inhibition with appropriate inhibitors for a defined culturing time.

2. The method according to claim 1, wherein the inoculating mixed culture step
(i) is obtainable by culturing a preculture of mixed microorganisms, preferably a mixed culture of fungi, adapted to solid or liquid, optimally inductive substrates cultivating on normal agar plates other "solid-state" (SSF) cultures, such as column reactors with inert carriers as a medium for supporting growth, or in any liquid cultures, such as shaking flasks or fermenter cultures as an inoculating culture for the subsequent main SSF cultures; and/or
(ii) is a culture which has run through an inductive preculture and one or more main cultures operated under selection pressure; and/or
(iii) is a culture which has run through an inductive preculture and one or more main cultures operated under selection pressure and is suitable to be employed for fermentation of the target substrate either directly or after preservation by freezing and/or lyophilization.

3. The method according to claim 1 or 2, wherein
(i) the appropriate selection pressure is built up and maintained by suitably selecting the culturing parameters, preferably selected from moisture content (water activity), pH value, temperature, oxygen availability, redox potential and nutrient composition; and/or
(ii) the inductive substrates and/or target substrates are selected from all kinds of raw or waste materials of natural (microbial, vegetable, animal or human)

and non-natural industrial origin and their mixtures, preferably the inductive substrate is selected from any plant-, animal- or microbial material and the target substrate is selected from any plant-, animal- or microbial material which have to be modified or degraded.

4. The method of claim 3, wherein the water activity is used for controlling the selection pressure, preferably the water activity is hold lower than one, preferably between 0.85 and 0.99, by the addition of water and its removal by means of temperature and suction.

5. The method according to any one of claims 1 to 4, wherein at least two microorganisms are employed for producing mixed cultures and precultures of mixed microorganisms, preferably

(i) fungi (ascomycetes, deuteromycetes) of the genera *Penicillium* spec., *Aspergillus* spec., *Trichoderma* spec., *Fusarium* spec., *Eurotium* spec., *Absidia* spec., *Neurospora* spec., *Mucor* spec., *Chaetomium* sp., *Rhizopus* sp. etc. are employed as microorganisms; or

(ii) fungi (ascomycetes, deuteromycetes) of the species *Penicillium chrysogenum*, *Eurotium amstelodami*, *Aspergillus niger*, *Aspergillus tubingiensis*, *Trichoderma harzianum*, *Trichoderma atroviride*, *Trichoderma reesei*, *Fusarium oxysporum* and *Neurospora intermedia* are employed as microorganisms; or

(iii) fungi (white rot fungi, brown rot fungi) of the genera *Trametes* spec., *Pleurotus* spec., *Phanerochaete* spec., *Nematoloma* spec. and *Agaricus* spec. etc. are employed as microorganisms, most preferably fungi (white rot fungi) which produce laccase or manganese peroxidase, such as organisms of genera *Trametes* spec., *Pleurotus* spec., *Phanerochaete* spec. and *Agaricus* spec. etc., are employed as microorganisms; or

(iv) fungi (white rot fungi) such as organisms of genera *Trametes* spec., *Pleurotus* spec., *Phanerochaete* spec. and *Agaricus* spec. etc. which, in addition to lignolytic enzymes, such as laccase or manganese peroxidase, secrete further oxidases which produce H_2O_2, such as glucose oxidases I and II (GOD), glyoxal oxidase, methanol oxidase, galactose oxidase, cellobiose

quinone oxidoreductase (CBQ) or cellobiose dehydrogenase (CDH) etc., are employed as microorganisms; or

(v) bacteria (actinomycetes) of the genus *Streptomyces* spec. etc. are employed as microorganisms;

most preferably the H_2O_2 required for the peroxidase action is added by metering.

6. The method according to any one of claims 1 to 5 which is performed in a continuous manner or in a step-wise manner with one or more process cycles.

7. The method according to any one of claims 1 to 6, wherein the continuously produced enzyme/substrate/fungus mixtures

(i) suitable applied as such, or after separation of the substrate/fungus mixture to obtain a liquid enzyme cocktail; and/or

(ii) are suitable to be used for the saccharification of all kinds of natural polysaccharide substrates or for the degradation of vegetable, animal or microbial polymers; and/or

(iii) are substituted by enzymes which are prepared by means of other methods or which are commercially available; and/or

(iv) are suitable for fermentation under essentially anaerobic or anaerobic conditions.

8. The method according to any one of claims 1 to 7, wherein the mixed cultures are suitable for the continuous production of specific hydrolase cocktails and/or oxidoreductase cocktails for processes target substrates, preferably in the wood-processing industry, paper and pulp industries, textile industry, leather industry, animal-processing industry, detergent industry, fodder industry, food industry, waste water, exhaust air and soil purification, in the processing of residual materials and in the processing of raw materials from naturally renewable resources.

9. The method according to claim 1 or 8, wherein the enzyme mixture is a hydrolytic/oxidative enzyme cocktail and is suitable

(i) for the enzymatic extraction (hydrolytic saccharification) of sugar beet chips at least by means of a two-phase culture; or

(ii) for the enzymatic extraction (hydrolytic saccharification, polymer degradation) of, for example, chemically pre-extracted materials, such as sugar cane, cereals and other vegetable, animal or microbial raw or waste materials; or

(iii) for the enzymatic extraction (hydrolytic saccharification, polymer degradation) of vegetable, animal or microbial raw or waste materials before a chemical and/or enzymatic and/or microbial treatment, such as special fermentations, or after them.

10. The method according to any one of claims 1 to 9 for producing an enzyme mixture (hydrolytic enzyme cocktails/oxidative enzyme cocktails) suitable for the enzymatic extraction (hydrolytic saccharification) of sugar beet chips or other polysaccaride containing material, wherein

(i) the inducer substrate is a rape extraction material; and/or

(ii) the microorganisms are *A. niger* and *A. tubigensis*; and/or

(iii) the water activity is initially set to be about 0.99; and/or

(iv) during the culture process *Neurospora intermedia* is added to the mixed culture and the water activity is reduced to about 0.96.

11. The method according to any one of claims 1 to 9 for producing an enzyme mixture (hydrolytic enzyme cocktails/oxidative enzyme cocktails) suitable for the enzymatic extraction (hydrolytic saccharification) of grass silage or other polysaccaride containing material, wherein

(i) the inducer substrate is a rape extraction material; and/or

(ii) the microorganisms are *A. niger*, *A. tugibensis* and *Neurospora intermedia*; and/or

(iii) the water activity is initially set to be about 0.98; and/or

(vi) during the culture process *Trichoderma atroviridae* and grass silage as substrate are added to the mixed culture and the water activity is raised to about 0.99.

12. The method according to any one of claims 1 to 9 for producing an enzyme mixture (hydrolytic enzyme cocktails/oxidative enzyme cocktails) suitable for the enzymatic extraction (hydrolytic saccharification) of corn silage or other polysaccaride containing material, wherein

(i) the inducer substrate is a rape extraction material; and/or

(ii) the microorganisms are *A. niger*, *A. tubigensis* and *Neurospora intermedia*; and/or

(iii) the water activity is initially set to be about 0.98; and/or

(iv) during the culture process *Aspergillus oryzae* and corn silage as substrate are added to the culture and the water activity is raised to about 0.99.

13. The culturing method according to any one of claims 1 to 12, wherein after optimum inoculation and selective process operation, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails)

(i) are either directly supplied to the downstream processes, such as special fermentations (methane fermentations), or first passed to a pre-hydrolysis container to effect a preliminary saccharification or, in the optimum case, a complete hydrolysis of the polysaccharides or other polymers, such as proteins and fats; or

(ii) are transferred to another solid state process operation in which the whole substrate which is to be fermented later is selectively utilized for producing enzymes and at least partially hydrolyzed.

14. The method according to claims 1 and 13, wherein

(i) said preinduced mixtures of microorganisms are mixtures of white rot fungi or mixtures of organisms which metabolize only low amounts of sugar at high enzyme forming rates; or

(ii) after optimum inoculation and selective process, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails), which were produced in a side stream in addition to the main solid-state process operation, are incorporated together with the main reaction into the subsequent fermentations by means of mixed populations of other microorganisms; or

(iii) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails) are transferred to another solid state process operation in which the whole substrate is selectively utilized for producing enzymes for composting purposes; or

(iv) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails) are transferred to another solid state process operation in which the whole substrate is selectively utilized for producing enzymes for the degradation of xenobiotics; or

(v) after optimum inoculation and selective process operation according to the invention, the preinduced mixtures of microorganisms and enzyme mixtures (hydrolytic/oxidative enzyme cocktails) are flowed through by liquid or gaseous induction substrates which are degraded or converted by the enzymes formed.

15. The method according to any one of claims 1 to 14, wherein the solid-phase cultures are generally performed in screw reactors, drum reactors, tower reactors, trickling film reactors, solid-state air-lift reactors, horizonatal mixers, vertical mixers etc. according to the principle of screw conveying, pressure screw conveying, conveying belt transport etc., optionally modified or in a cascade form, preferably the solid-phase cultures are performed

(i) in a screw reactor either singly or arranged in a cascade form; or

(ii) in special solid-state air-lift reactors; or

(iii) as batch cultures, fed-batch cultures or continuously.

16. The method according to any one of claims 1 to 15 which further comprises

conservation of the obtained mixed culture by decreasing the water activity during the fermentation process, preferably by air flow through the substrate or by a final drying step, preferably in a fluidised bed or belt dryer.

17. The method according to any one of claims 1 to 16, wherein a leaching of the produced enzyme containing solid (enzyme mixture) is carried out by moving or stirring it with water, buffer, detergent/water or detergent/buffer solutions, preferably in an amount of 1 to 10 or 1 to 20 by weight (enzyme containing solid to solution), for 30 min to 2hours and wherein the obtained enzyme slurry is filtered and the filtrate is further used as a solvent for additional leaching cycles (up to 10 times) for receiving a highly concentrated enzyme slurry.

18. An enzyme mixture and a metabolite mixture obtainable according to the method of any one of claims 1 to 17.

19. A fermentation method for processing one or more target substrates which comprises fermenting the target substrates with an enzyme mixture and/or metabolite mixture obtainable by the method of any one of claims 1 to 17.

20. A method for the conservation of enzyme-mixtures produced in solid state fermentation which comprises decreasing the water activity of the substrates during the fermentation process, preferably by air flow through the substrate; and or by a final drying step, preferably in a fluidised bed or belt dryer.

21. The method according to claim 20, wherein the enzyme mixture are produced by fungi, preferably by the fungi as defined in claim 5.

22. A method for the cultivation of microorganisms at equal growth rates by adjusting the water activity.

23. The method according to claim 22, wherein the cultivation of several microorganisms is controlled by sequential modification of water activity during the fermentation of solid substrates, allowing to cultivate two microorganisms at the same growth rate.

24. The method according to claim 22 or 23, wherein the cultivation of several pairs of microorganisms is controlled by

(i) sequential decrease of water activity during the fermentation of solid substrates, allowing to cultivate several appropriate pairs of microorganisms at the same growth rate; or

(ii) sequential increase of water activity during the fermentation of solid substrates, allowing to cultivate several appropriate pairs of microorganisms at the same growth rate.

25. Bioreactor, preferably for performing the culturing method according to one of the claims 1 to 24 , comprising

a fermentation module (10) for the fermentation of substrates under selection pressure whereby the fermentation module (10) comprises regulation means (28, 32) to adjust a fermentation environment,

a feeding means (16) being connected to the fermentation module (10) to feed the substrate,

an induction module (12) for adding reagents (agents conferring selection pressure) to the fermentation media,

a harvesting module (14) comprising outlet means and

a conveying means (24) to convey the media from the fermentation module (10) through the induction module (12) to the harvesting module (14).

26. Bioreactor according to claim 25 whereby the conveying means (24) is located within a common housing (22).

27. Bioreactor according to claim 25 or 26 whereby the regulation means comprises aeration means (32) and/ liquid feeding means (28).

28. Bioreactor according to one of the claims 25 – 27 whereby the aeration means (32) and/ or the liquid feeding means (28) are connected to a housing wall.

29. Bioreactor according to one of the claims 25 - 28 whereby the conveying means (24) comprises a conveying screw.

30. Bioreactor according to one of the claims 25 - 29 whereby the aeration means (38) and/ or a liquid feeding means are connected to a hollow shaft (36) of the conveying screw (24).

31. Bioreactor according to one of the claims 25 - 30 whereby the induction module (12) comprises an aeration means (46) and/ or a liquid feeding means.